

*CX*  
*CONT.*

for a time period sufficient to produce a culture having a compact multilayer like appearance whereby said culturing is performed in the absence of a feeder layer; and

(iii) identifying EG cells contained therein.

*SAC* > 14. (Twice Amended) An improved method of producing chimeric avians which comprises:

- (i) isolating primordial germ cells (PGCs) from an avian;
- (ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors;
- (1) leukemia inhibitory factor (LIF),  
(2) basic fibroblast growth factor (bFGF),  
(3) stem cell factor (SCF) and  
(4) insulin-like growth factor (IGF)

for a sufficient time to produce embryonic germ (EG) cells whereby said culture is maintained in the absence of a feeder layer;

(iii) transferring said EG cells into a recipient avian embryo; and

(iv) selecting for chimeric avians which have the desired EG phenotype.

*CX*

See the attached Appendix for the changes made to effect the above claim(s).

Please add the following new Claims 25-30:

*SJapM* > 25. An improved method of producing germline chimeric avians which comprises:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;  
(ii) maintaining [ ] such PGCs in a tissue culture medium containing at least the following growth factors;
- (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF);

(iii) transferring [ ] said PGCs into a Stage XII-XIV recipient avian embryo; and

(iv) selecting for germline chimeric avians which have [ ] the desired PGC phenotype.

C 3  
> 26. An improved method of producing germline or somatic cell chimeric avians

which comprises:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;  
(ii) maintaining [ ] such PGCs in a tissue culture medium containing at least the following growth factors;
- (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF),

for a sufficient time to produce embryonic germ (EG) cells;

- (iii) identifying and isolating embryonic germ (EG) cells from said [ ] cultured population of primordial germ cells;

- (iv) transferring said isolated EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (v) allowing said recipient avian to develop into a bird; and
- (vi) selecting for germline or somatic cell chimeric avians that express the PGC phenotype.

C3

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27. A method for producing avian embryonic germ (EG) cells comprising the following steps:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer sufficient to produce a culture having a compact multilayer like appearance; and
- (iii) identifying the EG cells contained therein.

28. A method for producing chimeric avians comprising:

- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
- (iii) transferring said purified PGCs into a recipient avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
- (iv) allowing said recipient avian to develop into a bird; and
- (v) selecting for chimeric avians that express the PGC phenotype.

29. A method for producing germline chimeric avians comprising:
- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
  - (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
  - (iii) transferring said purified PGCs into a recipient Stage XII-XIV avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
  - (iv) allowing said recipient avian to develop into a bird; and
  - (v) selecting for germline chimeric avians that express the PGC phenotype.
- C3*  
*cont.*

30. (New) A method for producing germline or somatic cell chimeric avians comprising:
- (i) isolating a pure population of primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
  - (ii) culturing said pure population of PGCs for a period of at least fourteen days in tissue culture;
  - (iii) identifying and isolating embryonic germ (EG) cells from said cultured population of primordial germ cells;
  - (iv) transferring said isolated EGs into a recipient Stage X avian embryo of the same species as the avian used to obtain said isolated, purified PGCs;
  - (iv) allowing said recipient avian to develop into a bird; and
  - (v) selecting for germline or somatic cell chimeric avians that express the PGC phenotype.